

Diagnosis of Protozoan Parasites in Livestock

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ABSTRACT

Livestock are an invaluable asset to agro-based countries like India, providing power, transport, fertilizer, fuel, and nutrition. They serve as a vital source of food and economic security for many families. However, protozoan parasites pose a significant threat to livestock health, adversely affecting their well-being, productivity, and overall contribution to rural livelihoods. Common protozoan genera affecting livestock include *Trypanosoma*, *Babesia*, *Theileria*, *Sarcocystis*, *Toxoplasma*, *Neospora*, *Giardia*, *Cryptosporidium*, and various species of *Coccidia*. These pathogens are associated with significant morbidity, mortality, and reproductive disturbances. Therefore, timely and accurate diagnosis, followed by appropriate treatment, is crucial to prevent disease progression, reducing transmission, and minimizing the impact on animal health and agricultural output. The objective of this article is to explore effective diagnostic methods for key protozoan diseases in livestock, facilitating early detection and intervention to curb their spread and mitigate their pathological consequences.

INTRODUCTION

Livestock forms the backbone of the Indian economy and is an essential part of daily life, contributing 4.11% to the national GDP and approximately 25.6% to the total agricultural GDP (Das *et al.*, 2020). Parasitic protozoans are considerably large in number, and thus the number of organisms they affect is also higher. These protozoans can cause significant morbidity and mortality in domestic animals and thus can constitute a substantial and serious grade of infectious diseases for almost all animal species including human, which in turn provokes a substantial economic loss. Some of these protozoan parasites are vector-borne diseases transmitted via arthropods as malaria, ticks as babesiosis and theileriosis, and flies as trypanosomosis and leishmaniasis. Such infections induce severe illness in infected animals, abnormal blood tests and may lead to death. Other protozoan diseases including toxoplasmosis and neosporosis possess great importance because of their capability for congenital transmission and inducing abortion, foetal resorption, mummification or delivery of foetus with congenital anomalies followed by death. While, other protozoa are causing highly contagious diseases in animals, and diarrhea is the main clinical form. These infections such as cryptosporidiosis, coccidiosis and amoebiasis are globally distributed and affect various animals.

Although there is an immediate need of controlling such protozoan infections regarding substantial economic losses and public health importance, but failed to do so because till date, potent vaccines or drugs are not available for most of protozoan infections in different animals. And often, most of these diseases are often neglected among communities in developing and developed countries. Some of the economically important protozoan diseases are listed below:

- 1) **Trypanosomosis:** Member of the genus *Trypanosoma* lives in the bloodstream and tissues of cattle, sheep, and goats in all of the world. These are flagellated protozoal agents that produce a variety of very serious disease of human and animals. The organism causes important diseases like Surra, Nagana, Chagas disease, Dourine etc.

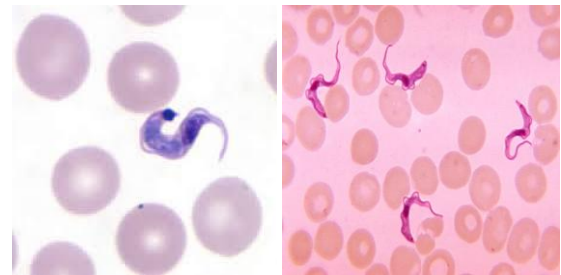





Fig 1: Trypanosoma spp. in blood smear

Diagnosis: Demonstration of trypanosomes in fresh blood smears from the infected animal is the surest way of diagnosing the acute infection since the organisms are readily demonstrable in blood smear. Thick and thin smear taken at the peak of fever may be positive for the parasites.

Chemical test:

- 1) **Mercuric Chloride test:**  A drop of test serum + 1 ml of 1:25,000 solution of mercuric chloride to be taken in a test tube, gently shake and mix.
 -  Appearance of white precipitate after 15 mins denotes the positive result. Limited to surra in camels having 1 month plus infection.
- 2) **Formal gel test (FGT)**  2 drops of concentrated formalin solution (37 % formaldehyde) + 1 ml of serum is mixed in a test

tube
✚ Coagulation of serum is found in positive cases which immediately turned white. It is reliable for camels.

3) **Thymol turbidity test:** ✚ 3ml Alkaline Thymol buffer + 1 ml test serum is mixed in a test tube. Immediate white turbidity with an increasing intensity upto 1 hr confirms the positive cases.

4) **Johne's nitric acid test:** ✚ 1 drop of test serum + 1ml of nitric acid is mixed in a test tube Yellow colour in about 1 hr indicates positive result.

5) **Stilbamidine test:** ✚ 0.5-0.25 ml of freshly prepared 10% aqueous solution of stilbamidine isothionate is taken in a small test tube. One drop of suspected serum is added on the surface of the above solution.

✚ Coagulated mass appears and begin to sink down in half a minute and dissolves in 5 to 10 minutes. Limited to surra in cattle.

Source: Bhatia et al., 2018

2) Babesiosis, caused by protozoan of the genus *Babesia* (Phylum: Apicomplexa), is an infectious tick- borne disease of livestock that is mainly characterised by fever, anemia, haemoglobinuria, icterus and weakness. Mostly *Boophilus (Rhhiphcephalus)*, *Haemaphysalis* etc. are responsible for transmission of the disease Infection of a vertebrate host is initiated by

inoculation of sporozoites form of parasites into the blood stream during the taking of a blood meal. Most common in adults and youngs above 1 year of age. *Babesia* affecting small ruminants are generally less pathogenic than large ruminants.

Diagnosis: Blood smears and clinical findings are useful in acute cases of piroplasmosis, but are not sufficient in subclinical cases. The complement fixation test is used as a serological test for bovine babesiosis. The most commonly used tests are ELISA, PCR and a DNA probe, which can detect specific parasitemias at very low levels of infection (Radostits, 2008). Recently, the 'reverse line blot' (RLB) is a versatile technique for simultaneous detection and identification of *Babesia* spp. Immunofluorescent antibody test (IFAT), is **OIE recommended** test for babesiosis.

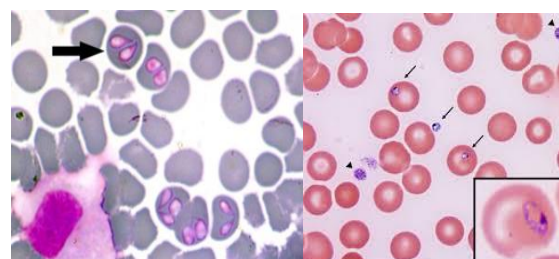


Fig 2: *Babesia* spp. in blood smear

3) Theileriosis, a hemoparasitic disease is caused by *Theileria* spp. in cattle, goats, sheep and wild and captive ungulates and are transmitted by ticks. These parasites, undergo repeated merogony in the lymphocytes ultimately releasing small merozoites, which invade the red cells to become piroplasms. Theileriosis cause infections ranged from clinically inapparent to rapidly fatal. The most common signs are pyrexia, anorexia, enlargement of superficial lymph nodes, nasal and ocular discharges and salivation. Constipation is recorded in some cases. Respiratory distress, coughing and pulmonary oedema are also seen.

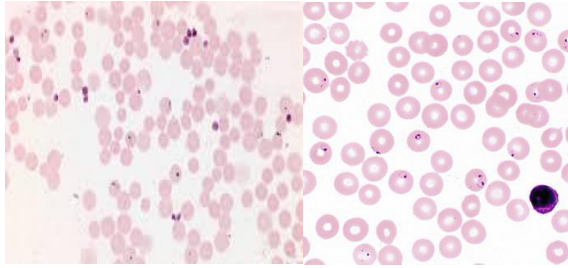


Fig 3: *Theileria* spp. in blood smear

Diagnosis: Investigation of Giemsa-stained blood smears and lymph node biopsy will reveal piroplasms in erythrocytes and schizonts in lymphocytes. For diagnosis, indirect fluorescent antibody test (IFAT) and indirect enzyme-linked immunosorbent assay (ELISA) are the most commonly used techniques. The ELISA test is more sensitive than IFAT.

- 4) Sarcocystosis, caused by *Sarcocystis* species is a disease of cattle, sheep and goats. Sexual reproduction phase occur in the intestine of a carnivore (dogs, cats) later, cysts settle in the muscles of cattle, sheep or goats. Sporocysts are shedded by carnivore's feces and then ingested by cattle, sheep, or goats. Then sporocysts hatch in the ruminant small bowel and invade the vascular endothelium during three phases of asexual reproduction. After third phase, merozoites enter the ruminant's muscle tissue and encyst as sarcocysts. clinical signs include fever, anorexia, anemia, weight loss, lameness, abortion and diarrhea (Radostits *et al.*, 2008). Neurologic signs are occasionally seen in cattle that include ataxia, tremors, muscular weakness, hypersalivation, blindness, opisthotonos and nystagmus (Smith and George, 2009). In infections that caused by *Sarcocystis bovicanis* in cattle there is usually loss of hair at the end of the tail

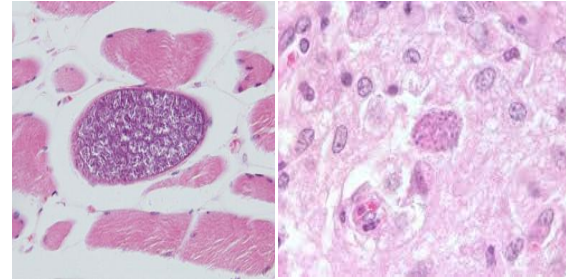


Fig 4: Tissue cyst in *Sarcocystis* spp. in muscle tissue smear

Diagnosis: Generally, Sarcocysts infections are diagnosed at meat inspection with grossly visible sarcocysts in the animal's muscle. When infection is very heavy in intermediate hosts, clinical signs and histological evidence of schizonts in the blood vessels of organs and the presence of cysts in the muscles at necropsy will be used for diagnosis

- 5) Neosporosis caused by *Neospora caninum* is a protozoan parasite of the phylum Apicomplexa of family Sarcocystidae. Dogs are the final host and sexual phase occur in them but, they are also intermediate host in prenatal infections. Cattle are the major intermediate hosts and asexual reproduction phase occur in them. In cattle, Neosporosis causes stillbirth, fetal resorption, mummification, abortion mainly between 5-7 month gestations and decreases in their milk production Fetus may born alive but congenitally diseased.

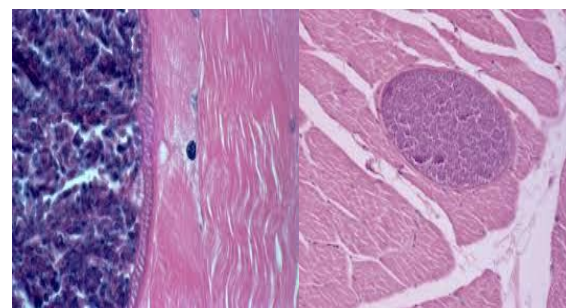


Fig 5: *Neospora* spp. in foetal tissue

Diagnosis: The diagnosis of neosporosis is based on the examination of maternal and fetal sera ideally combined with the examination of

fetal tissues. - Immunofluorescent antibody test (IFAT) and indirect enzyme-linked immunosorbent assay (ELISA) are used for diagnosis. - Histopathology of fetus: In histopathological examination of brain characteristic nonsuppurative encephalitis is suggestive of *Neospora* infection and also the lesions in the heart are characteristic for diagnosis

- 6) Coccidiosis occurs universally in young animals normally in calves between 3 weeks and 6 months of age. The pathogenesis of the disease mainly destroy crypt cells of the intestinal mucosa leading to bloody diarrhoea, tenesmus, abdominal pain, anorexia, dehydration and weight loss.

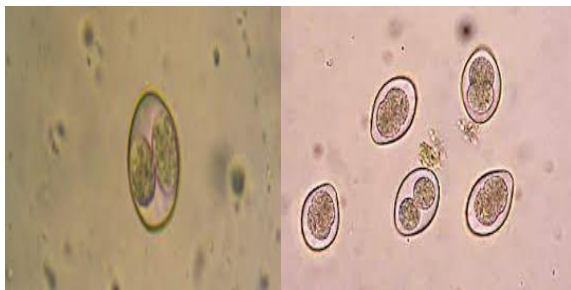


Fig 6: Oocyst of Coccidia spp. in faecal examination

Diagnosis is based on history, clinical and necropsy findings, and microscopic examination of feces. Acute coccidiosis can be diagnosed by direct examination of feces but in chronic coccidiosis that has very low oocysts in faeces, direct examination of faeces may not be adequate

- 7) Cryptosporidiosis spp. are intracellular protozoan parasites causing gastrointestinal disease and diarrhoea, and are important for young ruminants. In contrast to other protozoal agents, cryptosporidia do not require faecal excretion for sporulation to infective stages, and they sporulate in the intestine, characterised by anorexia, depression, weight loss, diarrhea, dehydration, high morbidity and possibly

death because of dehydration. *Cryptosporidium parvum* can cause diarrhoea in calves 5 to 15, and in lambs and kids 5 to 10 days of age.

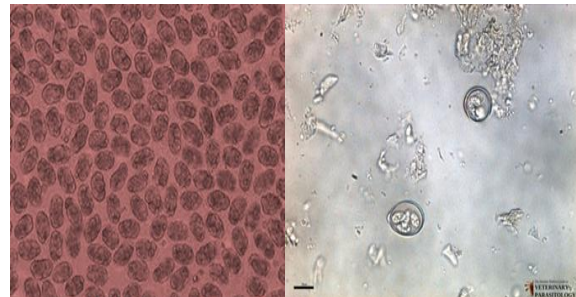


Fig 7: Oocyst of Cryptosporidium spp. in faecal examination

Diagnosis: Oocysts can be detected using Ziehl-Nielsen-stained fecal smears. Cryptosporidia can be diagnosed by fecal flotation. A number of molecular and immunological assays are available for diagnosis for example immunofluorescence (IF) or enzyme-linked immunosorbent assays (ELISA)

- 8) Giardiasis is caused by flagellate intestinal protozoan causing diarrhoea, weight loss, poor condition and lethargy. The parasite is a noninvasive and colonizes the mucosal surface of the small intestine.

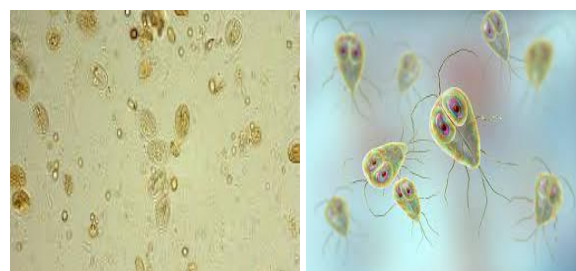


Fig 8: Trophozoites of Giardia spp. in faecal examination

Diagnostic methods for findings of *Giardia* include direct microscopy, fluorescent antibody staining, ELISA, and PCR

- 9) Besnoitiosis, a emergent parasitic disease that is caused by *Besnoitia besnoiti* in cattle, and *Besnoitia caprae* in goats of

family Sarcocystidae and an obligate intracellular apicomplexan protozoan parasite. They mainly develop to form bradyzoite cysts in fibroblasts in the dermis, subcutaneous tissues, fascia, nasal and laryngeal mucosa. After developing cysts in the skin, painful subcutaneous swelling, thickening of the skin, alopecia, and necrosis can be occur.



Fig 9: *Besnoitia* spp. in subcutaneous tissue

Diagnosis: Besnoitiosis can be diagnosed by demonstration of bradyzoites in skin biopsy smears or scleral conjunctival scrapings.

- 10) Toxoplasmosis, a systemic coccidian, a universal parasite that causes rarely abortion in cattle but is a major abortifacient in sheep and goats. Clinical toxoplasmosis in cattle is associated with fever, dyspnea, nervous signs and abortion and in small ruminants, particularly in sheep clinical signs include fever, abortion, prenatal mortality in lambs, generalized tremor, and dyspnea.



Fig 10: *Toxoplasma* spp.

Diagnosis is based on the clinical signs, presence of the characteristic small white

necrotic foci in cotyledons. ELISA test is also used to detect antibodies in fetal and maternal serum. Indirect fluorescent antibody test can be used for fluid collected from the fetal thorax or abdomen. Serological examination of dam can be used for diagnosis.

CONCLUSION:

Protozoan infections are a serious threat to the livestock sector hampering its health and productivity. The presence of protozoa in adult can also be a important sources of infection for newborn animals immediately after calving/lambing/kidding, due to the relaxation of immunity. Proper diagnosis and treatment is necessary for preventing the spread of diseases.

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